Innovative Food Science and Emerging Technologies 23 (2014) 10–17

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

journal homepage: www.elsevier.com/locate/ifset

High hydrostatic pressure processing of beef patties: Effects of pressure level and sodium tripolyphosphate and sodium chloride concentrations on thermal and aggregative properties of proteins

F. Speroni [a,b], N. Szerman [b,c], S.R. Vaudagna [b,c,*]

[a] Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA), CCT La Plata, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Calle 47 y 116, CP 1900 La Plata, Argentina
[b] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina
[c] Instituto Tecnología de Alimentos (ITA), Centro de Investigación de Agroindustria (CIA), Instituto Nacional de Tecnología Agropecuaria (INTA), CC77, B1708WAB Morón, Argentina

ARTICLE INFO

Article history:
Received 12 December 2013
Accepted 31 March 2014
Available online 13 April 2014

Editor Proof Receive Date 5 May 2014

Keywords:
High hydrostatic pressure
Beef proteins
Thermal behavior
Low sodium content
Sodium tripolyphosphate

ABSTRACT

Beef patties added with sodium tripolyphosphate (STPP; 0, 0.25 or 0.5%) and/or NaCl (0, 1 or 2%) were treated at 200 or 300 MPa (5 min, 5 °C) or kept refrigerated (non-pressurized). In non-pressurized patties, NaCl-solubilized proteins were denatured, whereas STPP-solubilized proteins remained in native state. At 200 MPa, myosin head was more sensitive to high hydrostatic pressure (HHP) than actin. 1% NaCl favored HHP-induced denaturation of myosin head and actin, whereas 0.25% STPP protected against that effect. At 300 MPa, STPP favored HHP-induced denaturation of myosin head, actin and other proteins. The effect of STPP at 200 MPa may depend on the presence of specific binding sites for STPP anion, which would be destroyed at 300 MPa. Insoluble aggregates were formed at 300 MPa in samples without salts. Salts minimized protein aggregation was observed at 300 MPa. Noticeable differences in thermal and aggregative behavior occurred whether HHP level was 200 or 300 MPa.
Industrial relevance: Currently, the reduction of sodium content in the manufacture of meat products is a hot topic and it is expected that this issue will become more relevant in the next years, as response to consumers' demands. Soluble high hydrostatic pressure-denatured beef proteins may provide interesting texture and technological properties to meat products with reduced salt content.

1. Introduction

Since the demand for healthy products has significantly increased in the last years, food manufacturers are interested in developing new products that respond to these demands. Traditionally, NaCl and sodium tripolyphosphate (STPP) are additives used in the manufacture of meat products. These additives are involved in the extraction and solubilization of myofibrillar proteins, which form a gel upon heating and led to a compact and uniform structure with improved water-retention (Desmond, 2006; Feiner, 2006; Sun & Holley, 2011; Tornberg, 2005). However, NaCl is associated with hypertension (Chen & Trout, 1991; Ruusunen & Puolanne, 2005) and polyphosphates are currently perceived as negative among consumers because of the clean labeling issue (Yusop, O'Sullivan, & Kerry, 2011).

During the last decade, several meat products treated by high hydrostatic pressure (HHP) such as pork and turkey cooked ham, dry-cured pork ham, prosciutto, sausages, marinated turkey or pork meat, ready-to-eat meats have been commercialized in Europe, Japan and North America (Sun & Holley, 2010). The main objective of the application of HHP in those products is the inactivation of pathogen microorganisms (such as *Listeria monocytogenes* and *Salmonella* spp.) and the extension of shelf-life. In addition, HHP processing causes physicochemical changes in meat proteins, such as depolymerization of F-actin, dissociation of actomyosin, solubilization of myofibrillar proteins and even their aggregation at pressures between 100 and 300 MPa (Buckow, Sikes, & Tume, 2013; Iwasaki, Noshiroya, Saitoh, Okano, & Yamamoto, 2006; Macfarlane, 1985). Those changes, which depend on the characteristics of the system and the conditions of processing (Fernández-Martín, Cofrades, Carballo, & Jiménez-Colmenero, 2002), improve meat binding properties and partially compensate the reduction of NaCl and STPP concentrations (Monahan & Troy, 1997). Several authors studied the application of HHP treatments (100–350 MPa), before or after, for the manufacture of low sodium content meat products (Crehan, Troy, & Buckley, 2000; Ferrari, Szerman, Sanow, Sancho, & Vaudagna, 2012; O'Flynn, Cruz-Romero, Troy, Mullen, & Kerry, 2014; Sikes, Tobin, & Tume, 2009; Villamonte, Simonin, Duranton, Chéret, & de Lamballerie, 2013). The application of HHP

* Corresponding author. Tel.: +54 11 4621 0446; fax: +54 11 4621 2012.
E-mail address: svaudagna@cnia.inta.gov.ar (S.R. Vaudagna).

http://dx.doi.org/10.1016/j.ifset.2014.03.011
1466-8564/

increased hardness values of different meat products such as beef and pork batters and patties (Ferrari et al., 2012; Sikes et al., 2009; Villamonte et al., 2013). Villamonte et al. (2013) concluded that this hardening effect was associated with the denaturation of myofibrillar proteins and the formation of a new protein component in pork batters. These authors also observed a synergic effect of NaCl and STPP on water binding capacity, enhanced by HHP. Moreover, Sikes et al. (2009) obtained similar cooking weight losses in beef batters formulated with 1% NaCl and HHP-treated at 200 MPa and those formulated with 2% NaCl and non-pressurized. However, we found that cooking weight loss increased when pressure level increased from 100 to 300 MPa in beef patties with low NaCl content (1% NaCl; 0.25% STPP) (Ferrari et al., 2012). Although the effects of the addition of NaCl and STPP on myofibrillar proteins have been largely studied (Barbut & Findlay, 1991; Findlay & Barbut, 1992; Kijowski & Mast, 1988; Paterson, Parrish, & Stromer, 1988; Pighin, Sancho, & Gonzalez, 2008; Xiong, Lou, Wang, Moody, & Harmon, 2000), the effects of those salts in combination with HHP processing on the solubilization and aggregation of myofibrillar proteins are still not well understood. Since some heterogeneous results were reported, a molecular characterization will provide a better understanding of the system.

The aim of this study was to evaluate the combined effect of salt concentration (NaCl and STPP) and HHP pressure levels on thermal and aggregative properties of beef proteins.

2. Materials and methods

2.1. Materials

Fresh beef shoulder clods (muscles *trapezius*, *deltoideus*, *latissimus dorsi*, *infraspinatus*, *triceps brachii*, *anconeus internus*, *anconeus extens*, *teres major* and *tensor fasciae antebrachii*) were obtained from a local market (COTO CICSA, Buenos Aires, Argentina). Meat pieces were vacuum-packed and stored at 1.0 ± 1.0 °C for 48 h. Muscles were defatted, and fat was conserved for patty preparation. After that, the pH of the pieces was measured using a puncture electrode (TESTO model 230, Sparta, NJ, USA), and those with normal pH (between 5.4 and 5.7) were selected. Then, meat and fat were vacuum-packed in Cryovac BB2800CB bags (permeability to: O_2 30 cm^3 m^{-2} 24 h^{-1} bar^{-1}; CO_2 150 cm^3 m^{-2} 24 h^{-1} bar^{-1}; water vapor 20 g 24 h^{-1} m^{-2}; Sealed Air Co., Buenos Aires, Argentina) and refrigerated at 1.0 ± 1.0 °C for 24 h.

The salts used were NaCl (Dos Anclas, Buenos Aires, Argentina) and STPP (N 15-16 Chemische Fabrik Budenheim R.A. Oetker, Budenheim, Germany).

2.2. Product manufacturing

Patties were prepared with the following composition: lean meat, 80% (w/w); fat, 10% (w/w); water, 10% (w/w) and NaCl (0, 1 or 2%) and/or STPP (0, 0.25 or 0.5%). The percentage of meat was modified according to the salt concentrations in the formulation. First, lean beef and fat were separately minced using a 4 mm plate in a Hobart meat grinder (Hobart Corp., Troy, Ohio, USA). During mincing, temperature was monitored using a puncture thermometer (Testo model 230, Sparta, NJ, USA), and it was lower than 8 °C during this step. After mixing lean meat and fat by hand, the mixture was minced through a 4 mm plate in a Hobart meat grinder (Hobart Corp., Troy, Ohio, USA). Then, STPP (dry powder) was added and manually mixed for 5 min. Finally, NaCl (previously dissolved in water at 8 °C) was incorporated and the mix was mixed by hand for 5 min. After that, portions of 140 g were formed into patties between grease proof papers using a manual patty press (100 mm diameter). Patties were stored at −20 °C for 24 h. Two smaller patties (50 mm diameter each) were obtained from each patty using a punch because the diameter of the HHP canister was 70 mm. After that, patties were vacuum-packed in Cryovac BB2800CB bags and stored at 1.0 ± 1.0 °C for 24 h. Henceforth, "control patties" corresponded to patties without salts non-subjected to HHP treatments.

2.3. High hydrostatic pressure treatments

Vacuum-packed patties were subjected to 200 or 300 MPa for a holding time of 5 min. HHP treatments were applied in a High Pressure System Stansted Fluid Power Ltd. model Iso-Lab FPG9400:922 (Stansted, United Kingdom), with a vessel working volume of 2 dm^3 (maximum working pressure: 900 MPa; temperature range: −20–120 °C). A mixture of propylene glycol and water (30:70) was used as compression fluid. Pressurization rate was 300 MPa min^{-1}. Conditioning temperature of vessel and initial temperature of compression fluid were 5 °C. The adiabatic heating induced an increase of fluid temperature that reached a maximum (10 °C) at 300 MPa. Patties (non-pressurized or HHP-treated) were stored at −40 °C for a maximum storage time of 4 months. Before testing, patties were thawed at 4 °C overnight.

2.4. Sample analysis

2.4.1. Protein content

The protein content of beef patties was determined by the Kjeldahl method (AOAC, 1990), using an N × 6.25 factor for calculation (2200 Kjeltec Auto Distillation, Foss Tecator, Hillerod, Denmark).

2.4.2. Thermal analysis

The thermal properties of beef patties were studied using a Perkin-Elmer Pyris-1 differential scanning calorimeter (Waltham, MA, USA). Indium was used as standard for temperature and heat flow calibration.

A sample of 19 to 24 mg of each patty, accurately weighed (Mettler Toledo H54, ±0.01 mg), was placed into an aluminum pan, which was hermetically sealed and equilibrated for 2 min at the initial scanning temperature. An empty pan was used as reference. The temperature increased from 20 to 90 °C at 5 °C min^{-1}. Changes of thermal denaturation enthalpy (ΔH) of total proteins were estimated as the area (heat flow vs. time) between the DSC curve and a straight line extended from the onset to the final temperatures of all detected transitions. The temperature of maximum heat absorption (Td) and the ΔH were determined by Origin Pro 8 software (Northampton, MA, USA); ΔH was expressed as J g^{-1} of protein. Peak Fit software V4.0 (Jandel Scientific Software, Chicago, IL, USA) was used to deconvolute the curves and calculate the percentage of ΔH corresponding to each individual transition. The ΔH values for myosin head and actin were reported because their identification and individualization was evident. The degree of denaturation was calculated as 100 multiplied by 1 minus the ratio between ΔH1 and ΔH2, where ΔH1 was the ΔH after HHP treatment and/or salt addition and ΔH2 was the ΔH for control or non-pressurized patties with salts addition. The degree of denaturation was calculated only in those cases where ΔH1 and ΔH2 were significantly different ($p < 0.05$). DSC analysis was carried out in quadruplicate for each treatment.

2.4.3. Salt-soluble protein extraction

Proteins were extracted from raw beef patties following the procedure of Wang, Smith, and Steffe (1990) with modifications. Extraction was carried out in a two-step procedure: the first step consisted in stirring (20 min at 4 °C) 0.2 g of chopped patty to 0.8 mL of 0.1 M NaCl, 0.05 M Na_2HPO_4/NaH_2PO_4 buffer, pH 6.5 (B0.1). Then, centrifugation was carried out (13,000 ×*g* for 15 min, 10 °C, Aircooled Microlitre Centrifuge Z 233 MK-2 Hermle, Gosheim, Germany). Supernatant, which contained proteins soluble in B0.1, was used for SDS-PAGE analysis. The second step of extraction consisted in exposing the pellet obtained after centrifugation to 0.8 mL of 0.6 M NaCl, 0.05 M Na_2HPO_4/NaH_2PO_4 buffer, pH 6.5 (B0.6) or to 0.8 mL of 0.6 M NaCl + 1% (w/v) SDS, 0.05 M Na_2HPO_4/NaH_2PO_4 buffer, pH 6.5 (B0.6-SDS). The second extraction was also carried out for 20 min at 4 °C followed by a centrifugation in the same conditions indicated above. The supernatants, which contained proteins soluble in B0.6 or B0.6-SDS, were used for SDS-PAGE analysis.